Protocol 9: Small-scale yeast transformation

1. Material

- Yeast overnight culture
- YPAD
- Carrier-DNA (10mg/ml)
- Polyethylene glycol 335 (50%)
- Dimethyl sulfoxide
- 1M/100mM LiAc
- Sterile H2O

2. Instruments

- Vortexer
- Heatblock
- Centrifuge (Haereus)

3. Experimental procedure

- Inoculate 3ml of YPAD and incubate overnight at 30°C and 220 rpm
- Inoculate 50ml of YPAD with overnight culture until OD600 reaches approximately 0.25-0.5
- Incubate 3-4h at 30°C on shaker.
- Pellet cells 5 min at 4000rpm and room temperature.
- Resuspend cells in 25ml sterile H2O.
- Centrifuge 5min at 4000rpm
- Resuspend pellet in 1ml 100mM LiAc and transfer to Eppendorf tube.
- Centrifuge 10 seconds and discard supernatant
- Resuspend pellet with 100mM LiAc to approx. 500µl
- Aliquot one 50µl fraction for each transformation.
- Centrifuge 10 seconds and discard supernatant.
- Add in this order:
    a. 240µl 50% PEG 3350
    b. 36µl 1M LiAc
    c. 5µl carrier DNA (10mg/ml)
    d. 2-5 µl plasmid
    e. 360µl with H2O
- Vortex about 1 min until pellet is resuspended
- Incubate 30 minutes at 30°C
- Heat shock: 20min at 42°C
- Centrifuge 10 seconds and resuspend pellet in 400µl H2O
- Plate 20-40µl

- Incubate 2-3 days at 30°C